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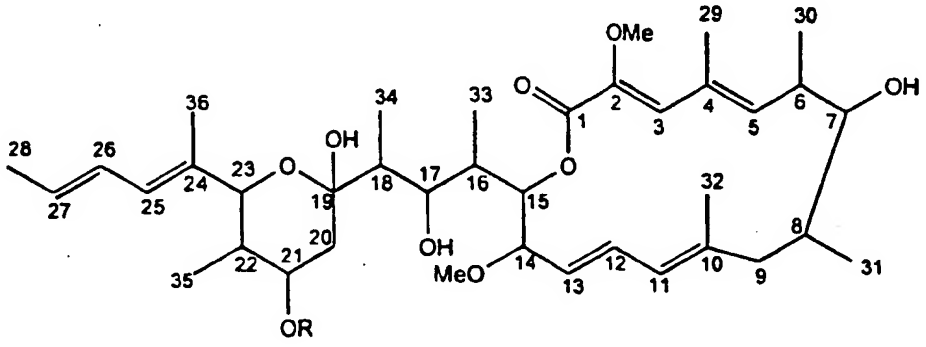
(54) Title: BAFILOMYCIN DERIVATIVES WITH ANTICANCER ACTIVITY



(II)

(57) Abstract: The invention provides compounds of formula (II), wherein R is -H or -CO-CH=CH-COOH, and pharmaceutically acceptable salts and esters thereof. Processes for the preparation of the compounds, pharmaceutical compositions containing them, and their therapeutic uses are also described. The compounds display activity against tumour growth in mammals. The invention further provides a method for the treatment or prophylaxis of tumour invasion by the administration of compounds of formula (II) or of known macrolide compounds.

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BAFILOMYCIN DERIVATIVES WITH ANTICANCER ACTIVITY

BACKGROUND OF THE INVENTION

Cancer mortality rates clearly indicate that no effective treatment exist for this group of diseases and so, the need for new anti-tumour compounds. Cancer is the result of two consecutive processes: the normal proliferation of a group of cells and the acquisition of a metastatic phenotype which allows some of them to grow in sites different from the original in the organism. Today, it is well established that cancer patients die due to metastasis. Cell invasiveness or invasion or surrounding tissues by tumour cells is a hallmark of the metastatic phenotype. Accordingly, a goal of the present invention is to provide new and known anti-cancer agents with a new anti-invasive activity suitable to be used to inhibit metastases formation.

Bafilomycins and hygrolidins are known macrolides (Wilton et al., 1985; Huang et al., 1984; Goetz et al., 1985; M.R.Boyd, 1989; Hensens et al., 1983; Suzukake et al., 1991; Kretschmer et al., 1985; Meyer et al., 1985; Werner et al., 1984; Seto et al., 1984; Corey et al., 1984) that have been found to inhibit tumour growth (US No. 5324720).

FIELD OF THE INVENTION

During a screening program we have found new macrolides with a bafilomycin-like structure with good anti-tumour activity. During this research also we have found that these new molecules as well as known bafilomycins displayed a remarkable inhibitory activity against invasiveness.

Novel bafilomycins can be isolated from culture broth of a new marine actinomycete strain. Production by aerobic fermentation under controlled conditions of the strain, and the isolation

and purification of these macrolides are described herein. Modification of these compounds as well as production also may be obtained by chemical synthesis.

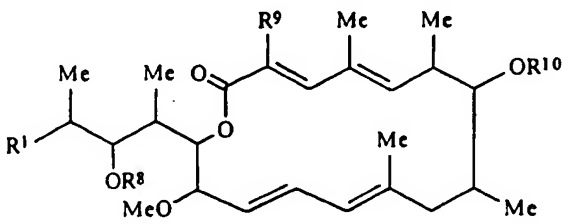
The use of compounds having at the same time significant antiproliferative activity against several human cancer cell types and inhibiting their metastatic behaviour when these tumour cells have it together benefits patient treatment in two possible ways, by killing the cells and by impeding those not dead to migrate and invade, as a necessary step to then grow in other parts of the body.

Yet another objective of this invention is to provide pharmaceutical compositions for administering to a patient in need of treatment the active compounds described herein.

SUMMARY OF THE INVENTION

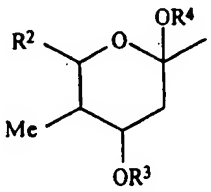
This invention provides with a new use of a family of known and new compounds with a macrolide-like structure with anti-tumour activity. These family of compounds display a very interesting anti-invasive activity together with its anti-tumour activity. In this invention the structure of some new family members as well as the process of obtaining some of them is also described, and the preferred process comprises cultivating a strain of a micro-organism capable of producing some of these macrolides in an aqueous nutrient medium with assimilable carbon and nitrogen sources and salts, under controlled submerged aerobic conditions. The compounds can be recovered and purified from the cultured broth.

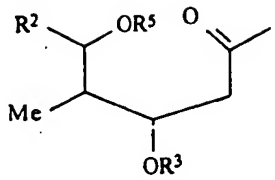
The family of new and known macrolides displaying a new activity against metastasis of mammal cancer cells with metastatic behaviour, have the following general formula (I):

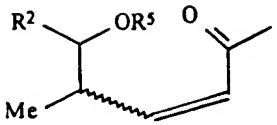


(I)

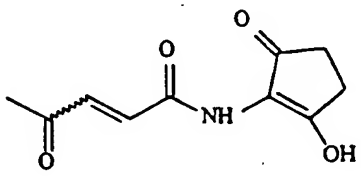
wherein R^1 is selected from the groups:

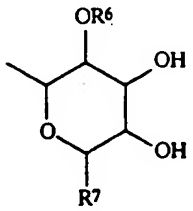






wherein R^2 is a straight or branched chain alkyl group having 1 to 12 carbon atoms or a straight or branched chain alkenyl or alkynyl group having 2 to 12 carbon atoms;
wherein R^3 is selected from the groups: H, CH_3, $COCH_3$, $CO(CH)_2CONH_2$, $CO(CH)_2COOH$,





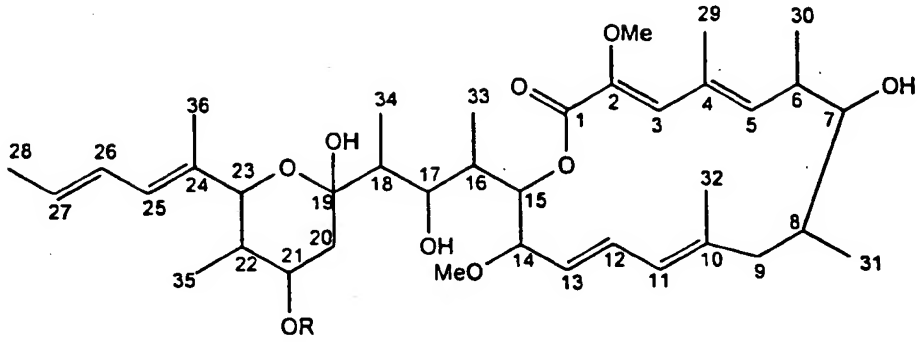
wherein R^6, R^4 are independently selected from the groups: H, CH_3
wherein R^7 is selected from CH_3, OH
wherein R^5, R^8, R^{10} are independently selected from H, $COCH_3$
wherein R^9 is selected from CH_3, OCH_3.

Therefore, the invention provides in a first aspect the use of compounds of formula (I), as defined above, or a pharmaceutically acceptable salt or ester thereof, in the manufacture of a medicament for the inhibition of tumour invasion in the treatment or prevention of cancer in a mammal.

The invention provides in a second aspect a method of treating cancer in a mammal by the inhibition of tumour invasion in said mammal, said method comprising administering to a mammal a compound of formula (I) as defined above or a pharmaceutically acceptable salt or ester thereof.

It is preferred that the amount of compound of formula (I) or pharmaceutically acceptable salt or ester thereof administered is less than that which is administered to inhibit tumour growth.

In another aspect, the invention provides the following new compounds of formula (II):



wherein R is a hydrogen atom or a group of formula CO-CH=CH-COOH, and pharmaceutically acceptable salts and esters thereof.

The compound of formula (II) wherein R is hydrogen is hereinafter known as IB-97227.
The compound of formula (II) wherein R is CO-CH=CH-COOH is hereinafter known as IB-98214.

There is also provided a process for the production of a compound of formula (II) as defined above or a pharmaceutically acceptable salt or ester thereof comprising cultivating a strain of a microorganism capable of producing a compound of formula (II), recovering the compound of formula (II) from the cultured broth, and, optionally, salifying or esterifying the recovered compound.

These new macrolides are active against mammal tumour cells and against metastasis.

Therefore, in a further aspect, the present invention relates to pharmaceutical preparations which contain as an active ingredient a compound of formula (II) as defined above or a pharmaceutically acceptable salt or ester thereof, as well as the processes for its preparation.

In a yet further aspect, the invention provides the use of a compound of formula (II) as defined above, or a pharmaceutically acceptable salt or ester thereof, in the manufacture of a

medicament for the treatment or prophylaxis of cancer in a mammal, in particular by the inhibition of tumour growth.

In a still further aspect, the invention provides a method for the treatment or prophylaxis of cancer in a mammal, comprising administering to a mammal in need of such treatment an effective amount of a compound of formula (II) as defined above or a pharmaceutically acceptable salt or ester thereof.

Known or new compounds described above can also be obtained by synthetic or semi-synthetic methods.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS:

In the formula (I) above, the group R^2 is a straight or branched chain alkyl group having 1 to 12 carbon atoms or a straight or branched chain alkenyl or alkynyl group having 2 to 12 carbon atoms.

As examples of the alkyl group, there may be mentioned the methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, s-butyl, t-butyl, n-pentyl, isopentyl, 2-methylbutyl, neopentyl, 1-ethylpropyl, n-hexyl, 4-methylpentyl, 3-methylpentyl, 2-methylpentyl, 1-methylpentyl, 3,3-dimethylbutyl, 2,2-dimethylbutyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,3-dimethylbutyl, 2-ethylbutyl, n-heptyl, 2-methylhexyl, 3-methylhexyl, n-octyl, 2-methylheptyl, 3-methylheptyl, 3-ethylhexyl, 1,1,3,3-tetramethylbutyl, n-nonyl, n-decyl, n-undecyl and n-dodecyl groups, of which alkyl groups having from 1 to 6 carbon atoms are preferred.

As examples of the alkenyl group, there may be mentioned the vinyl, 1-propenyl, 2-propenyl, 1-methyl-2-propenyl, 1-methyl-1-propenyl, 2-methyl-1-propenyl, 2-methyl-2-propenyl, 2-ethyl-2-propenyl, 1-butenyl, 2-butenyl, 1-methyl-2-butenyl, 1-methyl-1-butenyl, 3-methyl-2-butenyl, 1-ethyl-2-butenyl, 3-butenyl, 1-methyl-3-butenyl, 2-methyl-3-butenyl, 1-ethyl-3-

butenyl, 1-pentenyl, 2-pentenyl, 1-methyl-2-pentenyl, 2-methyl-2-pentenyl, 3-pentenyl, 1-methyl-3-pentenyl, 2-methyl-3-pentenyl, 4-pentenyl, 1-methyl-4-pentenyl, 2-methyl-4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl, 1-heptenyl, 2-heptenyl, 3-heptenyl, 1-octenyl, 2-octenyl, 3-octenyll, 1-nonenyl, 2-nonenyl, 3-nonenyl, 4-nonenyl, 1-decenyl, 2-decenyl, 3-decenyl, 1-undecenyl, 2-undecenyl, 3-undecenyl, 4-undecenyl, 1-dodecenyl, 2-dodecenyl, 3-dodecenyl and 4-dodecenyl groups, of which alkenyl groups having from 2 to 6 carbon atoms are preferred.

As examples of the alkynyl group, there may be mentioned ethynyl, 2-propynyl, 1-methyl-2-propynyl, 2-methyl-2-propynyl, 2-ethyl-2-propynyl, 2-butynyl, 1-methyl-2-butynyl, 2-methyl-2-butynyl, 1-ethyl-2-butynyl, 3-butynyl, 1-methyl-3-butynyl, 2-methyl-3-butynyl, 1-ethyl-3-butynyl, 2-pentynyl, 1-methyl-2-pentynyl, 2-methyl-2-pentynyl, 3-pentynyl, 1-methyl-3-pentynyl, 2-methyl-3-pentynyl, 4-pentynyl, 1-methyl-4-pentynyl, 2-methyl-4-pentynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, 5-hexynyl groups; 1-heptynyl, 2-heptynyl, 3-heptynyl, 1-octynyl, 2-octynyl, 3-octynyll, 1-nonynyl, 2-nonynyl, 3-nonynyl, 4-nonynyl, 1-decynyl, 2-decynyl, 3-decynyl, 1-undecynyl, 2-undecynyl, 3-undecynyl, 4-undecynyl, 1-dodecynyl, 2-dodecynyl, 3-dodecynyl and 4-dodecynyl groups, of which alkynyl groups having from 2 to 6 carbon atoms are preferred.

The new and known macrolides of the present invention may contain a carboxyl group and can therefore form salts by addition with a base. There is no restriction on the nature of such salts, provided that they are pharmaceutically acceptable, ie the salt must be about as active, more active, or not unduly less active than the free acid compound, and about as toxic, less toxic or not unduly more toxic than the free acid compound.

Preferred examples of salts include metal salts, for example, salts of an alkali metal such as sodium salts, potassium salts and lithium salts, salts of an alkaline earth metal such as calcium salts and magnesium salts, aluminum salts and iron salts; amine salts, for example, inorganic salts such as ammonium salts and organic salts such as t-octylamine salts, dibenzylamine salts, morpholine salts, glucosamine salts, phenylglycine alkyl ester salts, ethylenediamine salts, N-methylglucamine salts, guanidine salts, diethylamine salts, triethylamine salts, dicyclohexylamine salts, N,N'-dibenzyl-ethylenediamine salts, chloroprocaine salts, procaine

salts, diethanolamine salts, N-benzyl-phenethylamine salts, piperazine salts, tetramethylammonium salts and tris(hydroxymethyl)-aminomethane salts; aminoacid salts such as glycine salts, lysine salts, arginine salts, ornithine salts, glutamic acid salts and aspartic acid salts.

The new and known macrolides of the present invention may contain a hydroxy group and/or a carboxy group, and may therefore form esters. There is no restriction on the nature of such esters, provided that they are pharmaceutically acceptable, ie the ester must be about as active or not unduly less active than the free acid or alcohol, and about as toxic or not unduly more toxic than the free acid or alcohol.

Of the esters, the ester residue may be a conventional protecting group or a protecting group which can be cleaved *in vivo* by a biological method such as hydrolysis.

By "conventional protecting group" means a protecting group which can be cleaved by a chemical method such as hydrogenolysis, hydrolysis, electrolysis or photolysis.

When the ester is formed by acylating a hydroxyl group with a conventional protecting group, preferred examples of the conventional protecting group include aliphatic acyl groups having 1 to 10 carbon atoms such as formyl, acetyl, propionyl, butyryl, valeryl etc.; aromatic acyl groups such as benzoyl, 2-, 3- or 4-toluoyl, α- or β-naphthoyl; optionally substituted tetrahydropyranyl or tetrahydrothiopyranyl groups such as tetrahydropyran-2-yl, 3-bromotetrahydropyran-2-yl, 4-methoxytetrahydropyran-4-yl, tetrahydrothiopyran-2-yl and 4-methoxytetrahydrothiopyran-4-yl groups; optionally substituted tetrahydrofuranyl or tetrahydrothiofuranyl groups such as tetrahydrofuran-2-yl and tetrahydrothiofuran-2-yl groups; silyl groups, for example, trialkylsilyl groups such as trimethylsilyl, triethylsilyl, isopropyl-dimethylsilyl, t-butyldimethylsilyl, methyldiisopropylsilyl, methyl-di-t-butylsilyl and triisopropylsilyl groups and trialkylsilyl groups substituted with 1 or 2 aryl groups such as diphenylmethylsilyl, diphenylbutylsilyl, diphenylisopropylsilyl and phenyldiisopropylsilyl groups; alkoxymethyl or haloalkoxymethyl groups wherein the alkoxy or haloalkoxy moiety has from 1 to 4 carbon atoms, for example methoxymethyl, 1,1-dimethyl-1-methoxymethyl, ethoxymethyl, propoxymethyl, isopropoxymethyl, butoxymethyl and tert-butoxymethyl, 2,2,2-

trichloroethoxymethyl and bis(2-chloroethoxy)methyl groups; ethyl groups substituted with an alkoxy group having from 1 to 4 carbon atoms or a halogen atom such as 1-ethoxyethyl, 1-(isopropoxy)ethyl or 2,2,2-trichloroethyl groups; aralkyl groups wherein a methyl or ethyl group is substituted with 1 to 3 aryl groups (each of which may be optionally substituted with an alkyl group having from 1 to 4 carbon atoms, a haloalkyl group having from 1 to 4 carbon ataoms, an alkoxy group having from 1 to 4 carbon atoms, a nitro group, a halogen atom or a cyano group) such as benzyl, α-naphthylmethyl, β-naphthylmethyl, diphenylmethyl, triphenylmethyl, α-naphthyldiphenylmethyl, 9-anthrylmethyl, 4-methylbenzyl, 2,4,6-trimethylbenzyl, 3,4,5-trimethylbenzyl, 3,5-di(trifluoromethyl)benzyl, 4-methoxybenzyl, 4-methoxyphenyl-diphenylmethyl, 2-nitrobenzyl, 4-nitrobenzyl, 4-chlorobenzyl, 4-bromobenzyl, 4-cyanobenzyl or 1- or 2-phenylethyl groups; alkoxycarbonyl groups having from 2 to 7 carbon atoms such as the methoxycarbonyl and ethoxycarbonyl groups; alkenyloxycarbonyl groups having from 3 to 7 carbon atoms such as the vinyloxycarbonyl and allyloxycarbonyl groups; and aralkyloxycarbonyl groups wherein the aralkyl moiety is as defined above in relation to aralkyl groups.

When the ester is formed by esterifying a carboxyl group with a conventional protecting group, preferred examples of such a group include alkyl groups having from 1 to 6 carbon atoms such as those defined and exemplified above; alkenyl groups having from 2 to 6 carbon atoms such as those defined and exemplified above; alkynyl groups having from 2 to 6 carbon atoms such as those defined and exemplified above; haloalkyl groups having from 1 to 6 carbon atoms where the alkyl group is one of those defined and exemplified above and is substituted with a halogen atom; alkyl groups having from 1 to 6 carbon atoms wich are substituted with a hydroxy group such as 2-hydroxyethyl, 2,3-dihydroxypropyl, 3-hydroxypropyl, 3,4-dihydroxybutyl and 4-hydroxybutyl groups; alkyl groups having from 1 to 6 carbon atoms which are substituted with an aliphatic acyl group, such as acetylmethyl groups; aralkyl groups (as defined above); and silyl groups as defined above.

By "protecting group which can be cleaved *in vivo* by a biological method such as hydrolysis" means a protecting group which is cleaved *in vivo* by a biological method such as hydrolysis and forms a free acid or salt thereof, or a free alcohol. Whether an ester can in fact be cleaved in such a manner can be simply determined by administering it to an experimental animal,

such as a rat or mouse, by intravenous injection, examining the body fluid of the animal after administration and detecting the free acid or alcohol compound or a pharmaceutically acceptable salt thereof.

When the ester is formed by acylating a hydroxy group, preferred examples of the group which can be cleaved *in vivo* by a biological method such as hydrolysis include alkyl groups having from 1 to 6 carbon atoms which are substituted at the 1-position with an acyloxy group, for example the formyloxymethyl, acetoxymethyl, dimethylamino-acetoxymethyl, propionyloxymethyl, butyryloxymethyl, pivaloyloxymethyl, valeryloxymethyl, isovaleryloxymethyl, hexanoyloxymethyl, 1-formyloxyethyl, 1-acetoxyethyl, 1-propionyloxyethyl, 1-butyryloxyethyl, 1-pivaloyloxyethyl, 1-valeryloxyethyl, 1-isovaleryloxyethyl, 1-hexanoyloxyethyl, 1-formyloxypropyl, 1-acetoxypropyl, 1-propionyloxypropyl, 1-butyryloxypropyl, 1-pivaloyloxypropyl, 1-valeryloxypropyl, 1-isovaleryloxypropyl, 1-hexanoyloxypropyl, 1-acetoxybutyl, 1-propionyloxybutyl, 1-butyryloxybutyl, 1-pivaloyloxybutyl, 1-acetoxypentyl, 1-propionyloxypentyl, 1-butyryloxypentyl, 1-pivaloyloxypentyl and 1-pivaloyloxyhexyl groups, alkyl groups having from 1 to 6 carbon atoms which are substituted at the 1-position with a cycloalkylcarbonyloxygroup, the cycloalkyl part having from 3 to 8 carbon atoms, such as cyclopentylcarbonyloxymethyl, cyclohexylcarbonyloxymethyl, 1-cyclopentylcarbonyloxyethyl, 1-cyclohexylcarbonyloxyethyl, 1-cyclopentyl-carbonyloxypropyl, 1-cyclohexylcarbonyloxypropyl, 1-cyclopentylcarbonyloxybutyl and 1-cyclohexylcarbonyloxybutyl groups, and alkyl groups having from 1 to 6 carbon atoms which are substituted at the 1-position with a aromatic acyloxy group, such as benzoyloxymethyl groups; alkyl groups having from 1 to 6 carbon atoms which are substituted with alkoxycarbonyloxy groups having from 2 to 7 carbon atoms, such as methoxycarbonyloxymethyl, ethoxycarbonyloxymethyl, propoxycarbonyloxymethyl, isopropoxycarbonyloxymethyl, butoxycarbonyloxymethyl, isobutoxycarbonyloxy-methyl, pentyloxycarbonyloxymethyl, hexyloxycarbonyloxymethyl, cyclohexyloxy-carbonyloxymethyl, cyclohexyloxycarbonyloxy(cyclohexyl)methyl, 1-(methoxycarbonyloxy)ethyl, 1-(ethoxycarbonyloxy)ethyl, 1-(propoxycarbonyloxy)-ethyl, 1-(isopropoxycarbonyloxy)ethyl, 1-(butoxycarbonyloxy)ethyl, 1-(isobutoxycarbonyloxy)ethyl, 1-(tert-butoxycarbonyloxy)ethyl,

1-(pentyloxycarbonyloxy)ethyl, 1-(hexyloxycarbonyloxy)ethyl, 1-(cyclopentyloxycarbonyloxy)ethyl, 1-(cyclopentyloxycarbonyloxy)propyl, 1-(cyclohexyloxycarbonyloxy)propyl, 1-(cyclopentyloxycarbonyloxy)butyl, 1-(cyclohexyloxycarbonyloxy)butyl, 1-(cyclohexyloxycarbonyloxy)ethyl, 1-(ethoxycarbonyloxy)propyl, 2-(methoxycarbonyloxy)ethyl, 2-(ethoxycarbonyloxy)-ethyl, 2-(propoxycarbonyloxy)ethyl, 2-(isopropoxycarbonyloxy)ethyl, 2-(butoxycarbonyloxy)ethyl, 2-(isobutoxycarbonyloxy)ethyl, 2-(pentyloxycarbonyloxy)ethyl, 2-(hexyloxycarbonyloxy)ethyl, 1-methoxycarbonyloxy)propyl, 1-(ethoxycarbonyloxy)propyl, 1-(propoxycarbonyloxy)propyl, 1-(isopropoxycarbonyloxy)propyl, 1-(butoxycarbonyloxy)propyl, 1-(isobutoxycarbonyloxy)propyl, 1-(pentyloxycarbonyloxy)propyl, 1-(hexyloxycarbonyloxy)propyl, 1-(methoxycarbonyloxy)butyl, 1-(ethoxycarbonyloxy)butyl, 1-(propoxycarbonyloxy)butyl, 1-(isopropoxycarbonyloxy)butyl, 1-(butoxycarbonyloxy)butyl, 1-(isobutoxycarbonyloxy)butyl, 1-(methoxycarbonyloxy)pentyl, 1-(ethoxycarbonyloxy)pentyl, 1-(methoxycarbonyloxy)hexyl and 1-(ethoxycarbonyloxy)hexyl groups; and oxodioxolenylmethyl groups such as (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methyl, [5-(4-methylphenyl)-2-oxo-1,3-dioxolen-4-yl]methyl, [5-(4-methoxyphenyl)-2-oxo-1,3-dioxolen-4-yl]methyl, [5-(4-fluorophenyl)-2-oxo-1,3-dioxolen-4-yl]methyl, [5-(4-chlorophenyl)-2-oxo-1,3-dioxolen-4-yl]methyl, (2-oxo-1,3-dioxolen-4-yl)methyl, (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl, (5-ethyl-2-oxo-1,3-dioxolen-4-yl)methyl, (5-propyl-2-oxo-1,3-dioxolen-4-yl)methyl, (5-isopropyl-2-oxo-1,3-dioxolen-4-yl)methyl and (5-butyl-2-oxo-1,3-dioxolen-4-yl)methyl groups: optionally substituted phthalidyl groups such as phthalidyl, dimethylphthalidyl and dimethoxyphthalidyl groups: aliphatic acyl groups as described above; aromatic acyl groups as described above; half ester salt residue of succinic acid; phosphate salt residues; ester forming residues such as with amino acids; carbamoyl groups; carbamoyl groups substituted with 1 or 2 alkyl groups having from 1 to 6 carbon atoms; and 1-(acyloxy)alkyloxycarbonyl groups such as pivaloyloxymethyloxycarbonyl, of which the carbonyloxyalkyl groups are preferred.

When the ester is formed by esterifying a carboxy group, preferred examples of the carboxy-protecting group which can be cleaved *in vivo* by a biological method such as hydrolysis include alkyl groups having from 1 to 6 carbon atoms which are substituted by alkoxy groups having from 1 to 6 carbon atoms (which may further be substituted by a halogen atom or another alkoxy groups having from 1 to 6 carbon atoms), for example, the methoxyethyl, 1-ethoxyethyl, 1-methyl-1-methoxyethyl, 1-(isopropoxy)ethyl, 2-methoxyethyl, 2-ethoxyethyl, 1,1-dimethyl-1-methoxyethyl, ethoxymethyl, n-propoxymethyl, isopropoxymethyl, n-butoxymethyl, tert-butoxymethyl, 2-methoxy-ethoxymethyl, 2,2,2-trichloroethoxymethyl and bis(2-chloroethoxy)methyl groups; alkyl groups having from 1 to 6 carbon atoms which are substituted by a phenyloxy or naphthyloxy group such as phenoxymethyl groups and naphthyloxymethyl groups; alkyl groups having from 1 to 6 carbon atoms which are substituted by an alkoxycarbonyl group having from 2 to 7 carbon atoms such as methoxycarbonylmethyl groups; alkyl groups having from 1 to 6 carbon atoms which are substituted by a cyano group such as cyanomethyl and 2-cyanoethyl groups; alkylthiomethyl groups wherein the alkyl part has from 1 to 6 carbon atoms such as methylthiomethyl and ethylthiomethyl groups; arylthiomethyl groups such as phenylthiomethyl and naphthylthiomethyl groups; alkylsulfonyl alkyl groups wherein each alkyl part has from 1 to 6 carbon atoms and may optionally be substituted with a halogen atom, such as 2-methanesulfonylethyl and 2-trifluoromethanesulfonylethyl groups; arylsulfonylalkyl groups wherein the alkyl part has from 1 to 6 carbon atoms such as 2-benzenesulfonylethyl and 2-toluenesulfonylethyl groups; the above described 1-(acyloxy)alkyl groups; phthalidyl groups, as described above; the above-described aryl groups; the above-described alkyl groups having from 1 to 6 carbon atoms; carboxyalkyl groups such as carboxymethyl groups; and amide-forming residues of amino acids such as phenylalanine groups.

Pharmaceutical compositions which contain as an active ingredient a compound of formula (II) as defined above or a pharmaceutically acceptable salt or ester thereof (as described and exemplified above) are also covered by the present invention. Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) with suitable composition for oral, topical or parenteral administration, and they may contain each pure active compound or in combination with any

carrier or other pharmacologically active compounds. These compositions may need to be sterile when administered parenterally.

The correct dosage of a pharmaceutical composition of these macrolides will vary according to the particular formulation, the mode of application, and the particular *situs*, host and tumour being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

THE PRODUCING ORGANISM:

The micro-organism utilised for the production of these new macrolides is preferably an actinomycete strain, more preferably actinomycete strain ES20-008, a culture of which has been deposited in the Colección Española de Cultivos Tipo at the University of Valencia, Spain under the accession number CECT 3347. This deposit has been made under the provisions of the Budapest Treaty and all restrictions on the availability thereof to the public will be irrevocably maintained upon the granting of a patent on this application.

The organism was isolated from an unidentified marine sponge collected in Mediterranean waters.

All cultures were incubated at 27°C and records of results were made weekly up to 21 days.

A description of the organism is as follows:

Morphology:

The culture media utilized for this study were, ISP media No 2, 4, 5, 6 and 7 (Shirling and Gotlieb, 1966), ATCC medium No 172 (American Type Culture Collection Catalog, 1989), Czapek Agar (Atlas, 1993), Bennet Agar (Atlas, 1993), 1.5% Water Agar. All media were supplemented with 50% artificial sea water. After 21 days at 28°C growth was studied. Growth could only be detected on ISP-2, ISP-5, ISP-6, ISP-7, and Bennet's media. Several shades of orange were observed. No aerial mycelium was formed. Substrate mycelium was branched. No soluble pigment was observed.

Physiological characteristics:

For carbon and nitrogen utilization studies ISP-9 was used (Shirling and D.Gotlieb, 1966). Due to low growth rate of ES20-008 under defined media, the carbon and nitrogen utilisation tests showed residual growth so no clear results could be obtained.

NaCl resistance was determined by using ATTC's 172 medium containing increasing concentrations of NaCl. The optimal concentration of salt was around 1%. No growth was observed above 7% salt.

Cell chemical composition:

Aminoacids:

Diaminopimelic acid was determined by the method of Hasegawa et al.(1983). The *meso*-2,6-Diaminopimelic acid isomer was present in the whole cell hydrolysate of strain ES20-008.

Fatty acids:

FAMEs were determined by the method of Van der Auwera et al. (1986). The FAME composition as well as comparison with other strains representative of the Actnomicetales order is described in Table 1.

While the deposited organism is clearly preferred, the present invention is not restricted or limited to this particular strain or organisms. It is the intention of the present inventors to include any other producing organisms, strains or mutants within the scope of this invention.

TABLE 1

FAME composition of strain ES20 and other actinomycetes strains. Composition is given as percentage of total fatty acids content.

	13:0	i-14:0	14:0	i-15:0	a-15:0	15:0	i-16:1	i-16:0	16:1	16:0
ES20	< 1	< 1	2.01	< 1	< 1	1.98	< 1	< 1	4.35	34.73
STALBUS	< 1	6.52	< 1	9.88	22.92	< 1	5.50	25.29	< 1	3.75
SPAMETH	1.21	10.34	< 1	1.86	< 1	4.30	< 1	15.51	5.63	8.62
SPVIRIDO	< 1	4.04	1.10	18.94	2.71	4.89	< 1	26.44	< 1	4.43
AMCITRE	< 1	< 1	3.18	< 1	< 1	1.03	< 1	6.37	12.62	40
APBRAZIL	< 1	3.15	< 1	15.46	18.91	2.76	< 1	19.07	2.15	1.79
AMPDIGIT	< 1	11.57	< 1	11.21	9.96	< 1	2.87	34.23	< 1	1.08
AMYORIE	< 1	3.40	2.37	19.94	4.66	1.17	< 1	11.85	5.59	18.41
MNCHALC	< 1	1.68	< 1	8.91	2.29	1.53	1.15	38.23	< 1	1.88
MNECHCA	< 1	1.17	< 1	6.97	1.24	2.81	< 1	30.88	< 1	2.29
MNFUSCA	< 1	< 1	< 1	26.56	6.53	< 1	< 1	8.58	< 1	< 1
SACCAER	< 1	3.06	1.35	14.41	8.62	1.04	5.68	20.07	13.84	6.16
NOAFRI	1.51	5.43	3.35	4.62	< 1	7.46	3.09	22.18	2.69	5.15
MTSALMO	< 1	1.12	1.28	6.75	< 1	7.83	7.53	21.58	1.21	1.97
MTRUBRA	< 1	1.40	1.38	4.12	< 1	3.41	7.27	25.00	2.63	3.89
MTROSEO	2.03	3.65	5.14	3.86	< 1	9.03	3.02	12.31	3.46	6.95
AMROSEO	< 1	2.19	1.24	6.73	1.09	6.94	1.43	22.21	2.21	3.61
MTFERRU	1.03	1.91	1.19	1.94	< 1	6.43	4.12	21.50	2.32	2.34

	i-17:1	i-17:0	a-17:0	17:1	17:0	i-18:1	i-18:0	cis-18:1	18:0
ES20	< 1	< 1	< 1	5.75	2.32	< 1	< 1	22.24	2.49

STALBUS	1.28	3.38	8.60	< 1	< 1	< 1	1.09	< 1	< 1
SPAMETH	1.08	< 1	< 1	24.02	9.43	7.11	< 1	4.60	1.04
SPVIRIDO	< 1	2.60	1.58	11.36	8.58	7.48	< 1	< 1	1.16
AMCITRE	< 1	< 1	< 1	< 1	1.16	< 1	< 1	14.25	2.82
APBRAZIL	< 1	2.39	9.64	11.18	2.82	< 1	< 1	3.38	1.06
AMPDIGIT	< 1	1.28	5.08	4.39	1.64	< 1	1.76	7.60	1.54
AMYORIE	< 1	2.99	4.44	3.09	2.73	< 1	< 1	6.21	3.04
MNCHALC	1.49	2.32	2.25	5.43	6.95	14.58	1.31	1.28	2.68
MNECHCA	1.63	4.11	1.68	12.15	4.90	7.23	< 1	10.05	1.69
MNFUSCA	7.30	11.89	13.25	2.90	3.37	3.59	< 1	2.33	1.94
SACCAER	4.55	2.20	5.31	2.02	< 1	< 1	< 1	< 1	1.43
NOAFRI	2.35	< 1	< 1	8.15	4.75	17.03	< 1	< 1	1.23
MTSALMO	1.01	< 1	1.07	11.58	5.53	17.34	< 1	< 1	< 1
MTRUBRA	2.17	1.08	< 1	6.84	4.97	15.44	1.25	< 1	1.61
MTROSEO	1.17	< 1	< 1	13.51	4.46	18.67	< 1	1.77	< 1
AMROSEO	2.74	1.03	< 1	10.97	4.33	17.84	< 1	< 1	< 1
MTFERRU	< 1	< 1	< 1	23.51	5.71	12.15	1.27	1.43	< 1

ES20 = ES20-008; AMCITRE = *Actinomadura citrea* DSM 43461; AMPDIGIT = *Ampullariella digitata* ATCC 15349; AMROSEO = *Actinomadura roseoviolacea* DSM 43144; AMYORIE = *Amycolatopsis orientalis* DSM 40040; APBRAZIL = *Actinoplanes braziliensis* ATCC 25844; MNCHALC = *Micromonospora chalcea* ATCC 31395; MNECHCA = *Micromonospora echinospora calichinensis* NRRL 15839; MNFUSCA = *Micromonospora fusca* NRRL B-3298; MTFERRU = *Microtetraspora ferruginea* DSM 43553; MTROSEO = *Microtetraspora roseola* ATCC 33579; MTRUBRA = *Microtetraspora rubra* ATCC 27031; MTSALMO = *Microtetraspora salmonea* ATCC 33580; NOAFRI = *Nocardiopsis africana* DSM 43748; SACCAER = *Saccharothrix aerocolonigenes* NRRL B-3298; SPAMETH = *Streptosporangium amethystogenes* DSM 43179; SPVIRIDO = *Streptosporangium viridogriseum* ATCC 25242; STALBUS = *Streptomyces albus* DSM 40313

THE PRODUCTION STAGE:

Strain ES20-008 when cultured under controlled conditions in a suitable medium produces new macrolide antibiotics. This strain is grown in an aqueous nutrient medium, under aerobic and mesophilic conditions, preferably between 22°C and 35°C at a pH ranging between 6.0 and 8.0. A wide variety of liquid culture media can be utilised for the cultivation of the organism, useful media are those that include an assimilable carbon source, such as starch, dextrin, sugar molasses, glycerol, glucose and the like, an assimilable nitrogen source such as proteins, protein hydrolysates, defatted meals, corn steep, and the like, and useful

inorganic anions and cations such as sodium, magnesium, potassium, ammonium, sulphate, chloride, phosphate, carbonate, and the like. Trace elements may be added also. Aeration is preferably achieved by supplying air to the fermentation medium. Agitation is provided by a mechanical impeller. Conventional fermentation tanks have been found to be well suited for carrying out the cultivation of this organism. The addition of nutrients and pH control as well as antifoaming agents during the various stages of fermentation may be needed for increasing production and avoid foaming.

The required steps needed for production of these compounds by the preferred organism are:

Start with frozen or lyophilised mycelium. Obtain mycelial mass culturing the initial ceils in shake flasks with a culture medium containing some of the ingredients described above at mesophilic temperatures and in aerobic conditions, this step may be repeated several times, as needed, and the material collected will be used as an inoculum to seed one or several fermentation tanks with any appropriate culture medium, if desired these tanks can be utilised also as inoculum, and this step can be repeated several times when needed, or they can serve as the production stage, depending on the broth volume needed. The production stage can last from very few days to more than one week, depending on strain, inoculum stages, temperature and other conditions. Once the fermentation has reached its maximum yield can be harvested for the isolation of the new macrolides.

Production medium may be different than that used as inoculum. In Table 2 typical media are described that can be used for inoculum and production of these new macrolides:

TABLE 2

Inoculum medium (g/litre)		Production medium (g/litre)	
Dextrose	5	Dextrose	5
Starch	20	Dextrin	20
Beef extract	3	Soybean meal	3
Yeast extract	5	Yeast extract	5
Peptone	5	Peptone	1

$CaCO_3$	4	$CaCO_3$	4
NaCl	4	NaCl	5
Na_2SO_4	1	Na_2SO_4	2.5
KCl	0.5	KCl	0.5
$MgCl_2$	2	$MgCl_2$	0.5
K_2HPO_4	0.5	K_2HPO_4	0.5
		$(NH_4)_2SO_4$	0.5
Tap water to 1 000 ml			

Production of these compounds can be monitored by whole broth assay against murine leukaemia cells such as P-388 or any other sensitive cell or by HPLC or any other method with enough sensitivity.

ISOLATION OF NEW MACROLIDES:

Two new macrolide antibiotics IB-97227 and IB-98214 can be isolated from the mycelium cake by extraction with a suitable mixture of solvents such as $CHCl_3$:CH_3OH:H_2O. The activity is concentrated in the lower layer. The extracts from two repeated extraction can be combined and evaporated to dryness *in vacuo*.

Separation and purification of IB-97227 and IB-98214 from the crude active extract can be performed by the use of the proper combination of conventional chromatographic techniques.

Fractionation can be guided by the anti-tumour activity, or by TLC visualised with vanillin in conc. H_2SO_4, or analytical HPLC with photodiode-array detector. HPLC analysis are performed at room temperature using an analytical radial pack cartridge Resolve C18 (10m) using as mobile phase for IB-97227, methanol:0.025M phosphate buffer (pH 5.4) 85:15 and a flow rate of 2 ml/min. and plotted at 254 nm, in this conditions IB-97227 retention time is 3.06 min. The IB-98214 retention time is 2.34 min, using as a mobile phase methanol:water 95:5 in the same conditions described for IB-97227.

On the basis of detailed analysis of their various spectral characteristics, the pure compounds can be identified as IB-97227 and IB-98214.

The infrared absorption spectrum for IB-97227 in KBr, displays the following absorption maxima (ν_{max}): 3404, 3021, 2976, 1686, 1641, 1580, and 1445 cm^{-1}.

The UV spectrum for both IB-97227 and IB-98214 shows absorption (λ_{max}) at 240 and 285 nm.

The assignments of the ^1H and ^{13}C NMR spectra for IB-97227 and IB-98214 is given in Table 3 below. The ^1H NMR spectrum was recorded at 300 MHz and the ^{13}C NMR spectra at 75 MHz. The numbering of the carbons is as in the general formula (II) above. For IB-98214, carbon 1' is bonded to the oxygen atom of the remainder of the macrolide ring, the other carbons being numbered in sequence terminating with the carboxyl carbon (4').

TABLE 3

Position C/H No.	IB-98214		IB-97227	
	^{13}C shifts[a]	^1H shifts[a, b, c] ($CDCl_3$)	^{13}C shifts[a]	^1H shifts[a, b, c] ($CDCl_3$ + CD_3OD)
1	166.9		166.9	
2	141.4		141.3	
3	132.3	6.56 s, 1H	132.7	6.56 s, 1H
4	133.1		133.1	
5	142.5	5.74 d, 1H (J=9.0)	142.5	5.75 d, 1H (J=8.7)
6	36.5	2.51 m, 1H	36.6	2.49 m, 1H
7	81.0	3.29 m, 1H	80.9	3.25 m, 1H
8	40.3	1.92 m, 1H	40.2	1.86 m, 1H
9	41.3	1.90 m, 1H	41.2	1.90 m, 1H
		2.15 m, 1H		2.10 m, 1H
10	143.3		143.2	
11	125.2	5.79 d, 1H (J=10.6)	125.2	5.79 d, 1H (J=10.5)
12	133.1	6.51 dd, 1H (J=15.1, 10.7)	133.0	6.50 dd, 1H (J=14.7, 10.8)
13	127.1	5.14 dd, 1H (J=15.0, 9.4)	127.0	5.13 dd, 1H (J=15.0, 9.6)
14	81.8	3.88 dd, 1H (J=9.4, 8.8)	81.9	3.89 t, 1H (J=8.7)
15	76.3	4.96 dd, 1H (J=8.8)	76.3	4.90 d, 1H (J=8.4)
16	37.0	2.10 m, 1H	37.2	2.09 m, 1H
17	70.2	4.01 m, 1H	70.2	3.98 d, 1H (J=10.2)
		4.66 d, OH (J=4)		4.68 br s, OH
18	41.6	1.73 m, 1H	41.6	1.72 m, 1H
19	99.4		99.4	
20	43.2	1.18 m, 1H	39.4	1.24 m, 1H
		2.31 dd, 1H (J=11.9, 4.3)		2.33 m, 1H
21	70.8	3.74 m, 1H	74.6	4.99 td, 1H (J=11.0, 5.5)
22	40.9	1.39 m, 1H	37.9	1.60 m, 1H
23	79.9	3.94 d, 1H (J=10.5)	79.9	3.98 d, 1H (J=10.2)
24	133.1		132.5	
25	127.8	5.86 d, 1H (J=11.2)	128.3	5.85 d, 1H (J=11.1)

26	127.1	6.11 dd, 1H (J=14.7, 11.2)	127.1	6.10 dd, 1H (J=15.0, 11.0)
27	129.0	5.61 dd, 1H (J=14.9, 6.8)	129.1	5.57 dd, 1H (J=15.0, 6.9)
28	18.3	1.74 d, 3H (J=6.4)	18.3	1.73 d, 3H (J=6.0)
29	14.1	1.97 s, 3H	14.0	1.95 s, 3H
30	17.2	1.06 d, 3H (J=7.3)	17.1	1.05 d, 3H (J=6.9)
31	21.7	0.91 d, 3H (J=6.1)	21.7	0.90 d, 3H (J=5.4)
32	20.2	1.94 s, 3H	20.1	1.92 s, 3H
33	9.6	0.78 d, 3H (J=6.8)	9.6	0.78 d, 3H (J=6.6)
34	6.9	1.03 d, 3H (J=7.1)	6.8	0.99 d, 3H (J=6.9)
35	12.9	0.83 d, 3H (J=6.6)	13.0	0.65 d, 3H (J=6.0)
36	11.5	1.58 s, 3H	11.4	1.57 s, 3H
MeO(2)	59.5	3.45 s, 3H	59.5	3.43 s, 3H
MeO(14)	55.5	3.22 s, 3H	55.6	3.23 s, 3H
1'			166.3	
2'			130.1	6.58 d, 1H (J=15.9)
3'			140.2	6.82 d, 1H (J=15.9)
4'			173.2	

[a] Chemical shifts (δ) are in ppm
[b] Coupling constants (J) in hertz (Hz) are given in parentheses
[c] s: singlet; d: doublet; t: triplet; dd: doublet of doublets; m: multiplet; br: broad

BIOLOGICAL ACTIVITY:

All these new macrolides display good anti-tumour activity against several mammalian cancer cell lines. Their anti-tumour activity have been detected *in vitro* by culturing the tumour cells following the methodology described by Bërgeron, et al.[2] and by Schroeder, et al.[6]. Activity against several human tumour cell lines such as leukaemia, colon carcinoma, NSC lung carcinoma, melanoma, and the like has been observed.

To test the in vitro anti-invasive activity Transwell chambers (containing inserts) with basement membrane coated-porous filters were used. A naturally-produced basement membrane matrix (Matrigel) was allowed to dry under sterile conditions on porous filters (8.0 μm porosity) and invasive cells were then seeded on the filters. Following a 1-2 h period to allow their attachment to the Matrigel, cells were treated with the different compounds, extracts or fractions, or with vehicle alone for additional 24-48 h under appropriate conditions (37°C, 5% CO_2 atmosphere).

After the incubation period, the upper surface of the chamber containing the Matrigel and non-invasive cells was removed and the invasive cells attached to the lower surface of the porus filter were fixed and stained. Quantitation of invesive cells in control and drug-treated wells was made by estimating spectro-photometrically the cell-bound dye.

In order to define and separate specific effects on cell invasiveness with respect to the anti-growth activity of these macrolides, we employed in the invasion assays doses which have no cytotoxic/cytostatic effect on MDA MB-231 cells at the times analysed (24h). Thus, as can be seen in Table 4, a concentration of 0.1 µg/ml which do not affect cell proliferation lead to a 30% inhibition of invasiveness, indicating an invasion specific effect and so, the dual anti-cancer activity of these compounds.

Results showed a very significant migrating inhibition of these cells with these type of compounds.

The present invention will be further illustrated with reference to the following examples which aid in the understanding of the present invention, but which are not to be construed as limitations thereof. All percentages reported herein unless otherwise specified, are presented by weight. All temperatures are expressed in degrees Celsius. All incubations are carried out at 28°C and flasks are shaken in an orbital shaker. All media and recipients are sterile and all culture processes aseptic.

EXAMPLE 1:

Stock Culture: Whole broth of a pure culture of strain ES20-008 is preserved frozen in 20% glycerol.

Inoculum: A frozen culture or a well grown slant culture (5% vol.) is used to seed 100 ml of seed medium described previously contained in a 250 ml shake flask. The flask is incubated during 48 hr. 500 ml of the same medium in 2 l Erlenmeyer flask are seeded with 10% of the first stage inoculum. The flask is incubated during 48 h.

Fermentation: With 2.5 l of second stage inoculum seed 50 l of production medium already described in a 75 l fermentation tank. The fermentation is carried out during 96 hours with 400 rpm agitation and an air flow of 0.5 V/V.M.
Monitor secondary metabolite production by assay of whole broth against P-388 or by HPLC.

EXAMPLE 2:

Isolation: 10 litres of whole harvested broth were filtrated to separate the biomass and other solids. The mycelia cake was extracted twice with a mixture solvent (2200 ml) of $CHCl_3$: CH_3OH:H_2O (2:1:1), the activity was concentrated in the lower layer. The organic solvent was concentrated and evaporated to dryness *in vacuo* to yield 2.8 g of crude extract.

The extract was chromatographed on silica gel using a mixture of hexane/ethyl acetate and ethyl acetate/methanol as eluting solvents. 60 mg of a fraction A containing IB-98214 with anti-tumour activity were eluted with hexane/ethyl acetate 3:7, 300 mg of fraction B containing IB-97227 with anti-tumour activity were eluted with ethyl acetate/methanol 7:3.
Further purification of fraction A was achieved by column chromatography on silica gel and the activity (16 mg) was eluted with hexane/ethyl acetate 65:35. The last purification was carried out by column chromatography on C18 reversed phase using methanol / water 85:15 as the eluting solvent to yield 5 mg of pure IB-98214.
Fraction B is subjected to silica gel column chromatography using a mixture of chloroform/methanol 8:2 to yield 110 mg of active fraction. The final purification step was carried out by column chromatography on C18 reversed phase using methanol / water 85:15 as the eluting solvent to yield 25 mg of pure IB-97227.

EXAMPLE 3

Anti-tumour activity

The tumour cells employed have been P-388, A-549, HT-29, and MEL-28.

P-388 cells were seeded into 16 mm wells at $1x10^4$ cells per well in 1 ml aliquots of MEM 5FCS containing the indicated concentration of drug. A separate set of cultures without drug were seeded as control of growth to ensure that cells remained in exponential phase of growth. All determinations were carried out in duplicates. After three days of incubation at 37° in 10% CO_2 atmosphere with 98% humidity, the IC_{50} was calculated by comparing cell growth in the presence or absence of the drug.

A-549, HT-29, and MEL-28 cells were seeded into 16 mm wells at $2x10^4$ cells per well in 1 ml aliquots of MEM 10FCS containing the indicated concentration of drug. A separate set of cultures without drug were seeded as control of growth to ensure that cells remained in exponential phase of growth. All determinations were carried out in duplicates. After three days of incubation at 37° in 10% CO_2 atmosphere with 98% humidity, the cells were stained with 0.1% Crystal Violet. The IC_{50} was calculated by comparing the cell growth in presence and absence of drug.

In Table 4 the anti-tumour activity is expressed as IC_{50} in µg/ml.

TABLE 4

COMPOUND	CELL LINE : P-388	A-549	HT-29	MEL-28
IB-97227	0.001	0.001	0.001	0.002
IB-98214	0.06	0.025	0.025	0.25

Both compounds show a good growth inhibitory effect against different cancer cell lines

Anti-invasive activity

Materials: Transwell chambers: 6.5 mm diameter-8.0 µm porosity (Costar cat.# 3422)

Matrigel: basement membrane matrix: (Beckton-Dickinson, cat.# 40234 A)

Test, invasive cells: MDA-MB 231 (malignant human breast carcinoma cells)

Control, non-invasive cells: MCF-7 (human breast carcinoma cells)

Culture medium: Dulbecco's modified Eagles medium + 10% foetal calf serum (DMEM 10FCS).

Protocol: Transwell chambers were coated with 12.5 µg of reconstituted Matrigel and placed in 24-multiwell culture dishes containing 0.6 ml of culture medium in the lower compartment. 100.000 cells were seeded per well in a final volume of 0.1 ml of culture medium (upper compartment). The cells were allowed to attach to the Matrigel surface for 1-2 hours and then treated with different doses of test compounds or with vehicle alone for 24-48 hours at 37°C in a 5% CO2 atmosphere. After incubation period, the upper surface of the Transwell chamber containing the Matrigel and non-invasive cells was removed with the help of a

cotton-stick and the invasive cells attached to the lower surface of the porous filter were fixed in 1% glutaraldehyde for 10 min, washed three times in phosphate buffer saline (PBS), stained in 0.1% cresyl-violet solution for 30 min, washed three times in water, and allowed to dry at room temperature. When dried, the porous filters were cut-out, and the cell-bound dye extracted in 10% acetic acid and quantitated at 595 nm.

These results are expressed as percentage of control (non-treated) invasive cells in Table 5.

TABLE 5

Compound	Dose (μg/ml)	% Inhibition
IB-97227	0.1	30 %
IB-98214	0.1	36 %
BAF A1*	0.1	30 %
BAF B1*	0.1	30 %
BAF B2*	0.1	30 %

* BAF A1 = Bafilomycin A1, BAF B1 = Bafilomycin B1, BAF B2 = Bafilomycin B2

These data show inhibition at 24 hours after treatment at concentrations that do not affect cellular growth, therefore, the observed effect must only be due to an inhibition of invasiveness.

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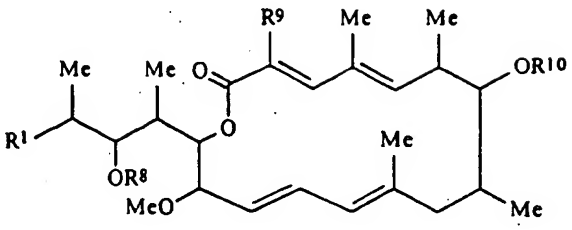
The present invention has been described in detail, including the preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of the present disclosure, may make modifications and/or improvements on this invention and still be within the scope and spirit of this invention as set forth in the following claims.

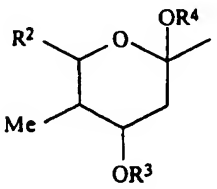
CLAIMS:

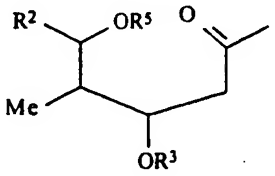
1. The use of a compound of formula (I):

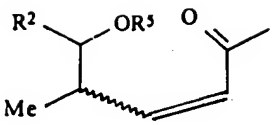


(I)

wherein R^1 is selected from the groups:

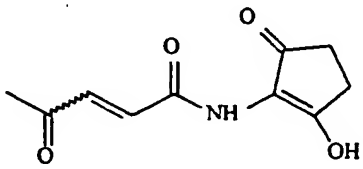


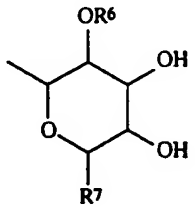




wherein R^2 is a straight or branched chain alkyl group having 1 to 12 carbon atoms or a straight or branched chain alkenyl or alkynyl group having 2 to 12 carbon atoms;

wherein R^3 is selected from the groups: H, CH_3, $COCH_3$, $COCH{=}CHCONH_2$, $COCH{=}CHCOOH$;





wherein R^6, R^4 are independently selected from the groups: H, CH_3

wherein R^7 is selected from CH_3, OH;

wherein R^5, R^8, R^{10} are independently selected from H, $COCH_3$

wherein R^9 is selected from CH_3, OCH_3;

or a pharmaceutically acceptable salt or ester thereof;

in the manufacture of a medicament for the inhibition of tumour invasion in the treatment or

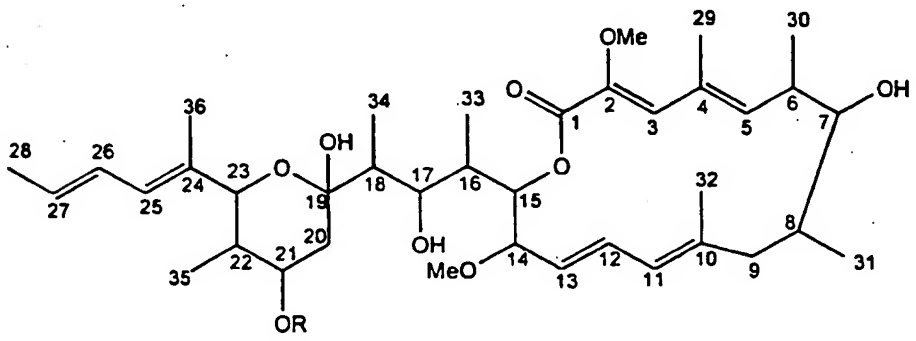
prevention of cancer in a mammal.

2. The use according to claim 1, wherein the amount of compound of formula (I) or pharmaceutically acceptable salt or ester thereof administered is less than that which is administered to inhibit tumour growth.

3. A method of treating cancer in a mammal by the inhibition of tumour invasion in said mammal, said method comprising administering to a mammal a compound of formula (I) or pharmaceutically acceptable salt or ester thereof as defined in claim 1.

4. The method according to claim 3, wherein the amount of compound of formula (I) or pharmaceutically acceptable salt or ester thereof administered is less than that which is administered to inhibit tumour growth.

5. A compound of formula (II):



(II)

wherein R is -H or -CO-CH=CH-COOH, or a pharmaceutically acceptable salt or ester thereof.

6. A compound according to claim 5, wherein R is hydrogen, or a pharmaceutically acceptable salt or ester thereof.

7. A compound according to claim 5, wherein R is -CO-CH=CH-COOH, or a pharmaceutically acceptable salt or ester thereof.

8. A process for the production of a compound of formula (II) as defined in any one of claims 5 to 7, or a pharmaceutically acceptable salt or ester thereof, comprising cultivating a strain of a microorganism capable of producing a compound of formula (II), recovering the compound of formula (II) from the cultured broth, and, optionally, salifying or esterifying the recovered compound.

9. A process according to claim 8, wherein the microorganism is an actinomycete strain.

10. A process according to claim 9, wherein the microorganism is the actinomycete strain ES20-008 (CECT-3347).

11. A pharmaceutical composition containing as an active ingredient a compound of formula (II) as defined in any one of claims 5 to 7, or a pharmaceutically acceptable salt or ester thereof, in conjunction with a pharmaceutically acceptable carrier or diluent.

12. A compound of formula (II) as defined in any one of claims 5 to 7 or a pharmaceutically acceptable salt or ester thereof for use as a medicament.

13. The use of a compound of formula (II) as defined in any one of claims 5 to 7 or a pharmaceutically acceptable salt or ester thereof in the manufacture of a medicament for the treatment or prophylaxis of cancer in a mammal.

14. The use of a compound of formula (II) as defined in any one of claims 5 to 7 or a pharmaceutically acceptable salt or ester thereof in the manufacture of a medicament for the inhibition of tumour growth in a mammal.

15. A method for the treatment or prophylaxis of cancer in a mammal, comprising administering to a mammal in need of such treatment an effective amount of a compound of formula (II) as defined in any one of claims 5 to 7 or a pharmaceutically acceptable salt or ester thereof.

16. The method according to claim 15, wherein the compound or composition is administered to inhibit tumour growth.

INTERNATIONAL SEARCH REPORT

Inter. ...nal Application No
PCT/GB 00/02511

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H17/04 A61K31/70 A61P35/04 C12P19/58 C12R1/04
C07D313/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H A61K A61P C12P C12R C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, MEDLINE, BIOSIS, CHEM ABS Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	JP 09 301991 A (MERCIAN CORPORATION) 25 November 1997 (1997-11-25) abstract; page 2, compounds I-3, I-4; page 6, table 1; page 7, table 2-4	1-4 5,11-16
A	Y.ISHIZUKA ET AL.: "Induction of Hydroxyapatite Resorptive Activity in Bone Marrow Cell Populations Resistant to Bafilomycin A1 by a Factor with Restricted Expression to Bone and Brain Neurochondrin." BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1450, 6 May 1999 (1999-05-06), pages 92-98, XP000900888 the whole document -/--	1,5, 11-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 August 2000	07/09/2000

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Intern. ...nal Application No
PCT/GB 00/02511

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 93 18653 A (MERCK & CO INC) 30 September 1993 (1993-09-30) page 7, compound 1; page 8, compound 6 ---	1,5
A	WILTON J H ET AL: "PD 118,576: A NEW ANTITUMOR MACROLIDE ANTIBIOTIC" JOURNAL OF ANTIBIOTICS,JP,JAPAN ANTIBIOTICS RESEARCH ASSOCIATION. TOKYO, vol. 38, no. 11, 1 November 1985 (1985-11-01), pages 1449-1452, XP000674511 ISSN: 0021-8820 page 1451, compound 2 ---	1,5
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Information on patent family members

Interi. ..nal Application No
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